Topical Botulinum Toxin

[a]ASHLEY COLLINS, MD; [b]ADNAN NASIR, MD
[a]Department of Biochemistry, North Carolina State University;
[b]Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina

ABSTRACT

Nanotechnology is a rapidly growing discipline that capitalizes on the unique properties of matter engineered on the nanoscale. Vehicles incorporating nanotechnology have led to great strides in drug delivery, allowing for increased active ingredient stability, bioavailability, and site-specific targeting. Botulinum toxin has historically been used for the correction of neurological and neuromuscular disorders, such as torticollis, blepharospasm, and strabismus. Recent dermatological indications have been for the management of axillary hyperhydrosis and facial rhytides. Traditional methods of botulinum toxin delivery have been needle-based. These have been associated with increased pain and cost. Newer methods of botulinum toxin formulation have yielded topical preparations that are bioactive in small pilot clinical studies. While there are some risks associated with topical delivery, the refinement and standardization of delivery systems and techniques for the topical administration of botulinum toxin using nanotechnology is anticipated in the near future. (*J Clin Aesthetic Dermatol.* 2010;3(3):35–39.)

Botulinum toxin is produced by the bacterium *Clostridium botulinum*. It is one of the most toxic proteins known, causing death by muscle paralysis.1,2 As a poison, it is most commonly encountered as a source of food poisoning, or Botulism.[1–3] There are seven serologically distinct but structurally similar types of botulinum toxin: A, B, C, D, E, F, and G.[1–5] They are each resistant to various physical and chemical agents. This paper will focus more on botulinum toxin A, the most commonly used in preparations for cosmetic uses.

In the early 19th century, Justinus Kerner (1786–1826) was the first to speculate about the potential therapeutic uses of botulinum toxin (BT) in muscle hyperactivity disorders.[3] The first therapeutic use of BT was proposed during the late 1960s by Alan B. Scott (Smith-Kettlewell Eye Research Institute). Edward J. Schantz (1908–2005) developed standardized BT preparations and safety standards for subsequent therapeutic use after performing extensive animal experiments.[2,3] In 1997, Scott was the first to therapeutically inject these BT preparations directly into overactive muscles to treat children with strabismus.[2,3] After the successful treatment of these patients, physicians began using focal injections of BT for other conditions involving overactive contraction of muscle.[2]

Facial lines and wrinkles appear due to a number of internal and external factors including sun exposure, loss of dermal elastic fibers, skin atrophy, and excessive muscle activity.[4] It is the repeated contraction of facial muscles that create dynamic wrinkles, producing expression in the face and neck. Lines and wrinkles are distressing to patients because they often are misinterpreted as signs of anger, fear, fatigue, melancholia, and aging. Patients often look to aesthetic medicine for resolution.

While treating blepharospasm in 1987, Said et al[5] observed that botulinum toxin type A (BoNTA) improved glabellar frown lines.[5,6] They noticed that their patients had a decrease in wrinkles around their eyes after therapy and were the first to conduct controlled studies on the use of BT for cosmetic purposes.

BoNTA decreases muscle activity by blocking overactive nerve impulses that trigger excessive muscle contractions.[7] Facial wrinkles, which are maintained by the contraction of small muscle fibers in the face, can be relaxed with small injections of the toxin. Glabellar furrows of the forehead (frown lines) and lateral canthal rhytids (crow's feet) were found to be the most responsive to the treatment.[2] Multiple studies have demonstrated BoNTA's safety and efficacy in wrinkle improvement.[5,6] The United States Food and Drug Administration (FDA) approved Botox® Cosmetic (Allergan, Inc., Irvine, California) in 2002 for the treatment of moderate-to-severe glabellar lines in patients 65 years or younger, and Dysport (Medicis Pharmaceutical

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ADDRESS CORRESPONDENCE TO: Adnan Nasir, MD; E-mail: anasir@pol.net

Corporation, Scottsdale, Arizona) for the same indication in 2009.[6]

BoNTA is a complex mixture of proteins containing botulinum neurotoxin coupled with various nontoxic proteins. Botulinum neurotoxin consists of a heavy chain and a light chain linked together by a single disulfide bond.[8] The neurotransmitter, acetylcholine (ACh), is released by a transport protein chain, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex.

The mechanism of action for BoNTA occurs in two phases: Phase 1, in which the nerve-muscle communication is blocked, and Phase 2, in which the nerve-muscle communication is restored. Phase I consists of three components: binding, internalizing, and blocking.

In binding, the heavy chain portion of BoNTA binds to the cell membrane of the motor nerve (glycoprotein structure) via an unidentified high-affinity "acceptor" molecule.[7,8] This high-affinity binding action allows for efficient uptake of BoNTA by the motor nerve and facilitates selective, targeted treatment at the injection site.[7]

Internalization of the neurotoxin occurs as the BoNTA protein molecule passes through the cell membrane of the motor nerve and into its cytoplasm through endocytosis. It is here that the enzymatic component (light chain) of the BoNTA protein molecule is activated.

Blocking of ACh then ensues as the light chain of the botulinum neurotoxin binds with high specificity to the SNARE protein complex.[8] The light chain specifically cleaves the SNAP-25 protein that normally enables vesicles storing ACh to attach to the cell membrane.[7,8] Cleaving the SNAP-25 protein prevents these vesicles from fusing with the membrane, interrupting the release of ACh into the neuromuscular junction. Thus, nerve impulses that control muscle contractions are blocked and muscle activity is decreased. Cleaving SNAP-25 has also been reported to block the release of neuropeptides involved in the transmission of painful sensations, theoretically reducing an individual's sense of pain.[8]

Nerve-muscle communication is restored in Phase 2 of the action mechanism.[7] The effect of BoNTA is temporary. Previous nerve-impulse activity is restored over the course of several months, depending on the individual patient and the condition for which he or she is being treated. Phase 2 consists of nerve sprouting and reestablishment of the original nerve connection.

During nerve sprouting, new nerve endings emerge and connect to the muscle after the original nerve ending is blocked, renewing the ability of the nerve to cause muscle contractions. Eventually, the new nerve sprouts retract and the original nerve ending regains its function, reestablishing the original nerve connection, and not permanently altering the long-term adequacy of the neuromuscular junction.

BoNTA is highly specific for motor nerve terminals and has a higher capacity for diffusion in muscle upon injection.[9] These factors make BoNTA an ideal agent for induction of muscle weakness and temporary paralysis. More importantly, BoNTA is not lethal to motorneurons; however, recovery from its effect is dose dependent until the neurotoxin has saturated all of the neuromuscular junctions in the treatment areas. In general, a lengthier recovery is observed following higher doses.

Secondary bone loss is one controversial but potential consequence of muscle paralysis. In a study conducted by Grimston et al,[9] a single dose of Botox was injected into the musculature around the knee joint of mice to induce significant paralysis of the injected limb for 3 to 4 weeks. The mice continued to use the injected limb for balance to move around the cage indicating that the effects of body weight on bone were still present. Botox treatments significantly reduced muscle mass compared to saline controls as measured 21 days post injection. Consequently, it was also shown that bone loss was demonstrated to be significant when compared to control animals after 24 days as measured by femoral cortical thickness and quantitative trabecular bone indices—bone volume/tissue volume (BV/TV). After an average of 7 to 8 weeks recovery from muscle, there continued to be differences between the BoNTA-injected leg and the noninjected leg 12 weeks post injection, suggesting bone loss was only partially reversible.[9] This type of bone loss is similar to that observed in humans exposed to acute disuse, such as spinal cord injury or bed rest.

PSYCHOSOCIAL ASPECTS OF BoNTA

Due to its proven efficacy and relative safety for more than 10 years, the use of BoNTA in cosmetic dermatology has increased in popularity.[5,10] In 2007, BoNTA injections were the top nonsurgical procedure with Americans spending almost $13.2 billion on cosmetic procedures overall.[10,11] Patients are now choosing a more subtle approach to improvements of the aging face than traditional cutting surgery.[10]

Research conducted among consumers concluded that many consumers are needle averse and would still prefer a more noninvasive alternative that would provide the same benefits as BoNTA injections.[12] In the United States, there are 11 million consumers in the target demographic for BoNTA treatments, but less than 10 percent have actually received treatment. Providing an attractive option to current injections would expand the market considerably. A safe, targeted alternative may be found in topical BoNTA.

The target consumer market can be organized into the following three categories: 1) past users of BoNTA injections who would like a less painful, cheaper option; 2) consumers with no past history of injections because of the expense and/or pain associated with them; and 3) consumers currently undergoing regular injections who would welcome a more comfortable way to maintain each treatment.[11]

Research demonstrates that the psychological profile of clients treated with BoNTA differs significantly from the profile of those using other noninvasive beauty products. BoNTA treatment is associated with greater anxiety due to

premorbid psychosocial problems triggered by the procedure (e.g., aversion and phobia in relation to needles and pain). This can be a particular problem in patients with a comorbid fear of being treated with a "deadly toxin." Patients also worry about the effects of the treatment and may be unprepared for the appearance-related changes after the treatment. Although they may know about the likely changes beforehand, individuals who rely heavily on demonstrative facial expressions (e.g., actors, politicians, salespersons) may be psychologically unprepared for the emotional distress of facial muscular paralysis. It appears that people are largely motivated to avoid the negative consequences of the aging process, and prepared to tolerate the psychological distress associated with the procedure to achieve their desired goal of "youth and beauty." The trends indicate that patients are satisfied with the outcome of these treatments as the effects directly contribute toward reducing the negative perceptions of aging. However, while the effects are welcome, the delivery of getting those effects is often less well received. For these clients, topical BoNTA has the potential to supply patients with similar results while eliminating the damaging psychosocial effects associated with a more invasive and direct procedure.

Injection therapy is not easily applicable to all skin areas, such as the throat, neck, hands, or around the mouth and eyes, as it is more cumbersome to disperse smaller quantities of the toxin over larger areas through injection techniques. Injections are also known to have a number of adverse events associated with the treatments including flu-like symptoms, headache, facial pain, redness at the injection site, respiratory infections, and possible muscle weakness. Some of these adverse events, however, are no higher than a placebo in controlled double-blind studies.

Topical BoNTA offers some solutions for many of the concerns associated with BoNTA injections. Though not a quantitative replacement for injection therapy, it is an effective alternative to those wanting cosmetic improvements of the face as well as treatment for hyperhidrosis without the pain associated with needles.[12] Topical formulations cause only a mild weakening; therefore, it is potentially safer for use in the areas where muscles are highly sensitive to smaller doses of the toxin.[11] A leading factor in the rationale behind developing a topical BoNTA cream is to eliminate the need for multiple traumatic injections for better comfort, or overinjection of the product, which, in unskilled hands, can leave a patient with several months of full paralysis to a treatment area. Topical treatments are also more convenient. One scenario for treatment includes self administration. Initial treatment could be administered in a clinic setting under strict medical supervision. Follow-up applications could potentially be done by the patient at home after appropriate instruction is given. This could limit follow-up office visits and would be a welcome cost savings to the consumer. However, the scenario of home-based, follow-up therapy is not without its hazards. Distributing BoNTA for general use in the hands of the public, after only modest in-office training, could lead to misuse of the product. For example, this could lead to incorrect application, application in nonindicated sites, and application by those to whom it was not initially prescribed and for whom it may be contraindicated.

NANOPARTICLE TECHNOLOGY AND BoNTA CREAM

Nanotechnology allows the potential to deliver molecules into the skin, which ordinarily do not penetrate the corneal layer.[15] This ability to enhance penetration of active ingredients offers many benefits. At least two companies, Transdermal Corp., Birmingham, Michigan, and Revance Therapeutics, Newark, California, have made sufficient progress on topical delivery of BoNTA for clinical trials. Transdermal Corp., formed in August 2008, has developed an FDA-approved topical BoNTA cream based on commercially viable ionic nanoparticle technology (InParT).[11] This transdermal noninvasive drug delivery technique preserves the bioactivity of molecules without denaturing them. The cream (CosmeTox) contains BoNTA and is intended for the softening of facial rhytides and a reduction in hyperhidrotic conditions. Active ingredients in the cream allow for greater toxin stability at room temperature for extended periods of time. Topical BoNTA allows for deeper delivery of the active molecules into the skin in order to encourage the same effect of injected BoNTA—inhibiting the release of ACh and blocking neuromuscular transmission.[11] The stabilized toxin cream is applied topically onto the skin and dosed by the quantity of cream applied. Using nanospheres and absorption enhancers, the toxin is delivered into the skin without any known skin damage or systemic toxicity. The cream also behaves in a similar fashion as an injectable in that when it is applied, it stays local for targeted delivery.[14] Smaller studies have demonstrated that the toxin is not present in the peripheral blood at measurable levels following application). In this way, treatment is truly needle free, pain free, and absent of distant toxin spread.

Several studies have been conducted on topical botulinum, each with promising results. Transdermal Corp. tested paralysis in mice using the stabilized toxin cream compared with injection to confirm that toxin potency was maintained in the cream when left at room temperature.[11] The control group was injected with toxin into the calf muscle of the hind leg and the experimental group was treated with the same size dose of cream on the same area. Test results showed quantitatively that the toxin cream elicited similar clinical response (induction of paralysis in the hind legs) when compared with the injected toxin, proving that there was no toxin degradation in the cream. Also, analysis of collected blood samples in this study demonstrated no detectable toxin in the blood.

A further study was also conducted to test the efficacy of topical BoNTA cream (CosmeTox) on facial wrinkles.[4] Only subjects with no prior history of toxin treatment were included. Researchers split the subjects into two randomly assigned groups, treating one with BoNTA cream and the other with a placebo cream containing no

toxin. The study involved 4 to 7 weeks of treatment with 12 weeks of follow-up evaluation. Within the first two weeks of treatment, the BoNTA group reported subjective improvements in their appearance, including less wrinkling, but also noticed a fading of dark circles under their eyes as well as a hyperpigmentation reduction in treatment areas. The active ingredient cream was compared to a placebo on 40 female subjects. The active ingredient cream contained a concentration of 2U/mL BT and the vehicle control contained no BT. Subjects were treated nightly for 4 to 7 weeks on the face, chin, and neck areas and followed for 12 weeks. They were assessed by Facial Line Outcomes and Self Perception of Age questionnaires. The Facial Lines Outcome scores were improved and maintained for the duration of the study period of almost three months. The Self Perception of Age was also reduced in the majority of subjects. According to the authors, placebo arm subjects reported no effect on any measure. Subjects also reported they looked younger than their current age. At Week 4, more than 85 percent of the BoNTA cream group rated their wrinkles as improved by at least 75 percent. In contrast, the placebo group saw no significant changes in their appearance. Atamoros[16] studied 77 subjects (61 women, 16 men) with moderate-to-severe lateral canthal lines using RT001 (Revance Therapeutics), a proprietary combination of 150kD BoNTA coupled with a peptidyl macromolecule transport system. This peptidyl transport system binds BoNTA through electrostatic interactions and reportedly permits transepidermal flux of toxin via cell-to-cell macropinocytosis. RT001 reportedly permits delivery of BoNTA to the dermis without altering its activity. Atamoros randomized subjects and treated each lateral canthal area with a single, 30-minute application of 0.5cc of varying doses of BoNTA versus placebo. Subjects were followed for up to 28 days after therapy and assessed for lateral canthal line severity at rest and after a smile on a four-point scale of rhytides (absent, mild, moderate, severe). Subjects in low-dose (0.5–4.5ng of botulinum toxin) and high-dose (6–10.5ng of botulinum toxin) cohorts achieved reductions in lateral canthal line scores. A greater proportion of subjects in the higher dose cohort achieved two-point reductions in lateral canthal line scores. Adverse events were noted in 63.6 percent of subjects and included involuntary muscle contractions (29.9%) and application-site mild-to-moderate erythema (23.4%). Ocular irritation was noted in the low-dose cohort. Localized ocular burning, stinging, itching, foreign body sensation, dryness, and erythema were reported. No cranial nerve abnormalities were noted. No gender subgroup analyses were reported.

Topical botulinum treatment has also been tested in patients suffering from hyperhidrosis. Hyperhidrosis is a substantial problem, affecting up to three percent of the world's population—7.5 million in the US alone.[12,17] The current injectable BT requires up to 40 injections to treat the underarms as well as many uncomfortable injections to treat the hands or feet. Topical anesthesia and/or nerve blocks are often used with these injectable botulinum toxin treatments, as these areas have much higher sensory innervation and are exquisitely sensitive, particularly the hands and feet. In addition, injections in the palms and soles are subject to complications involving the underlying motor function of the hands and feet that can effect one's daily activities. In another Transdermal Corp. study of hyperhidrosis, subjects without prior BT treatments were treated with Topical Toxin-A cream for 4 to 7 weeks and 16 weeks of follow up.[11] In this open-label, treatment-only study, 20 healthy subjects (10 men, 10 women) were pre-screened for hyperhidrosis by gravimetric or starch-iodine testing. Subjects were treated in the study clinic for their first visit and observed for one hour before discharge. Subjects then received individual treatment packs of Toxin-A (1cc containing 3 units) for home therapy. Home therapy was once daily. Subjects returned to the clinic after three days for assessment of sweating by the starch-iodine test. The results were favorable, with underarm sweating reduced or stopped in three applications. On the palms and soles, sweating decreased 80 to 90 percent in five applications. A 90-percent improvement was maintained throughout the subjects' 16-week follow-up evaluation. This study indicated that topical botulinum toxin was able to elicit an effect of the function of the eccrine glands, typically located in the mid-reticular dermis.[12] Adverse events included local tingling sensations, redness at the site, and excessive dryness of the skin where the cream was applied.[11] Glogau[17] conducted a small study of 12 subjects with axillary hyperhidrosis using RT001. Two-hundred units of BoNTA cream was applied topically and subjects were assessed for axillary hyperhidrosis using the quantitative Minor Starch Iodine assay and found to have a 65-percent reduction in sweat activity versus 25-percent reduction in sweat activity for placebo at four weeks. No systemic adverse events were noted. Folliculitis, eczema, tenderness, and erythema were observed.

CONCLUSION

Overall, several studies have demonstrated the efficacy of topical BoNTA for cosmetic treatment of facial rhytides and for hyperhidrosis. While these studies are small and proprietary, results from two independent manufacturers show that the topical toxin is stable at room temperature, reduces fine lines and wrinkles with multiple applications, and reduces sweating patterns in patients with palmoplantar hyperhidrosis. The topical formulations are well tolerated by both men and women without long-standing adverse events. Most importantly, subjects prefer the idea of topical treatment to injections. The potential impact that a topical BoNTA product can have on the cosmetic and clinical arena is very compelling. One potential concern is inadvertent or deliberate misuse. Misapplication could lead to ptosis or expressive facial asymmetry. Patients could also share cream with friends or colleagues for whom it is contraindicated. These

limitations will be a source of debate as to how and where these topical products are used and prescribed.

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